Interval mapping of growth quantitative trait loci in Japanese Black beef cattle using microsatellite markers and half-sib regression analysis

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ABSTRACT

A confirmatory scan for chromosomal regions of bovine chromosome 1 segregating QTL influencing birth weight, weaning weight, yearling weight, preweaning and postweaning average daily gains was performed by genotyping half-sib progeny of four Japanese Black sires using microsatellite DNA markers. Data were analysed by generating an F-statistic every 1cM on a linkage map by the regression of phenotype on the probabilities of inheriting an allele from the sire after adjusting for the fixed effects of sire, sex, parity and season of birth as well as age as a covariate. Permutation tests at chromosome-wide significance thresholds were carried out over 10,000 iterations. A significant QTL for birth weight at 114 cM was detected in Sire 2 Family. This identification of a birth weight QTL in Japanese Black Cattle would be useful for the implementation of marker-assisted selection.

KEYWORDS: Growth, Japanese Black Cattle, microsatellite markers, QTL

INTRODUCTION

Breeding beef bulls are evaluated based on their own growth rate and yearling weight because of the positive correlation between growth and carcass traits (Koch et al. 2004, Baik et al. 2003). Japanese Black is the main beef cattle breed in Japan and is revered as a producer of high quality meat. The growth of this breed as reflected by liveweight changes from birth to yearling age is important for the early attainment of slaughter weight. Identifying quantitative trait loci (QTL) has the potential to significantly increase the rate of genetic improvement through implementation of marker-assisted selection (MacNeil and Grosz, 2002). For traits that are difficult or expensive to measure, are lowly heritable, occur late in life or are determined post-mortem, marker-assisted selection may increase the rate of response relative to selection based on estimated breeding value alone (Davis and DeNise, 1998). Therefore, selection indices that include QTL with accurately estimated effects on growth traits could reduce the amount of lengthy and costly data collection by providing a means of genetic evaluation early in the life cycle.

Microsatellite DNA markers on genetic maps are used to identify inheritance patterns of linked segments of the genome in structured pedigree populations. Significant associations of marker allele with the phenotype of interest suggest linkage of the markers to QTL. The detection and mapping of QTL for birth weight, liveweight, growth and carcass traits in paternal half-sib families of Charolais x Brahman, Brahman, Piedmontese, Belgian Blue and Beefbooster cattle has been reported (Davis et al. 1998, Stone et al. 1999, Casas et al. 2000, Li et al. 2002 and Casas et al. 2003, Li et al. 2004a, Li et al. 2004b). The mapping of QTL is the first step towards the identification of genes and causal polymorphisms for traits of importance in agriculture (Seaton et al., 2002). Collaborative studies on QTL mapping for carcass traits in Japanese Black cattle between the Livestock Improvement Association, Shirakawa Institute of Animal Genetics and twenty-one Prefectures in Japan have been on-going (Mizoguchi 1998). From these collaborative studies, some preliminary results have been reported at conferences. A

number of simulated and theoretical work on marker-assisted selection (Saito and Iwaisaki 1996, 1997a, 1997b, Saito et al. 1998) as well as mathematical modelling of QTL cluster effects in granddaughter design, multi-group and outbred populations (Matsuda and Iwaisaki 2000, 2001a, 2001b, 2001c) have been published. Therefore in this study, we report for the first time, results of QTL analysis for growth traits in 112 half-sib progeny from four Japanese Black sires. Preliminary genome-wide scanning in our laboratory using only 30 animals (unpublished data) had suggested Bos taurus autosomes (BTA) 1, 2 and 5 as chromosomes containing segregating QTL significantly influencing growth traits in Japanese Black cattle. Stone et al. (1999) had also reported a significant QTL affecting birth weight on BTA1 in Brahman x Hereford cattle. More recently, Kim et al. (2003) detected a significant QTL for yearling weight on bovine chromosome one (BTA1) in Angus x Brahman crosses. Comparative mammalian genomics reveal that BTA1 is equivalent to the human chromosome 3 (http://bos.cvm.tamu.edu/htmls/rhbov1.html) which has been demonstrated to harbour growth-regulating genes such as growth hormone secretagogue receptor also known as ghrelin (Hosoda et al. 2003, Shuto et al. 2002), glycogenin (Mu et al. 2001) and Pit-1 (Ohta et al. 1992, Hendriks-Stegeman et al. 2001). It is therefore justifiable to focus on BTA 1 in the scan for growth QTL in Japanese Black cattle. Our objective in this study was to conduct a confirmatory scan for segregating QTL on BTA1 influencing body weights at birth, weaning, yearling and average daily gain before and after weaning by genotyping more Japanese Black cattle.

MATERIALS AND METHODS

Animals and management: One hundred and twelve paternal half-sib progeny of four Japanese Black sires produced by artificial insemination at the Department of Livestock and Grassland Science, National Agricultural Research Centre for Western Region, Oda, Shimane Prefecture, Japan, were genotyped for this study. Routine management of the animals involved recording of weight at birth and monthly thereafter, until 18 months of age. Calves were allowed

to suckle their dams in addition to being fed 1.5 kg/day/head of concentrate and 1 kg/day/head of corn silage until five months of age when they were weaned. After weaning, they were moved to the grower's barn and still raised on concentrates (37% corn grain, 39% rice bran, 17% soybean meal, 7% minerals) and corn silage until 10 months of age. Between 10 and 18 months of age, they were moved to another barn and fed intensively. The proportions of the ration on dry matter basis were: 61% corn grain, 34% soybean and corn glutein meal, 2% bran and 3% mineral. For every 20kg bag, this ration provided an estimated 21% crude protein, 3.5% crude fat, 5% crude fibre, 7% ash, 0.6% calcium, 0.40% phosphate and a total digestible nutrient of 77%. From 18 to 24 months of age, breeding females were returned to the calving barn while steers were moved to the fattening barn and raised primarily on "Mosa meal" a specially formulated fattening ration containing 77% corn and rye grain, 10.5% wheat and rice bran, 9% soybean oil meal and 3.5% mineral supplement. At all ages, routine veterinary vaccinations and health checks were observed.

Extraction of genomic DNA: Following the method of Sambrook et al. (1989) and described in detail elsewhere (Malau-Aduli et al. 2003), genomic DNA was extracted and prepared from blood leucocytes and sperm.

Polymerase chain reaction (PCR): PCR pre-mix (13 µl) that comprised of: 10.55 µl of distilled water, 1.04 µl of 2.5 mM dNTP Mixture (Takara, Shiga, Japan), 1.3 µl of 10 x buffer containing 15 mM $MgCl_2$ and 0.11 µl of 25 mM of $MgCl_2$ was prepared. A primer (12.5 pmol/ µl) of microsatellite DNA markers each of which was labelled with one of three different fluorescent labels, FAM, HEX and TET supplied by the Shirakawa Institute of Animal Genetics, Fukushima, Japan, based on the bovine genetic map at the U.S. Meat Animal Research Centre (Kappes et al., 1997; http://sol.marc.usda.gov) was added to the PCR pre-mix. Genomic DNA (1 µl) (conc of 20ng/µl) was added followed by 0.5 µl of Taq polymerase enzyme (conc of 0.75

units/μl) containing 50% glycerol (Takara, Japan). The PCR plates were hotplate -sealed and subjected to PCR in a DNA thermal cycler. The annealing temp settings were: 50ºC, 55ºC and 60ºC.

Genotyping: Multiplex genotyping was carried out. Prior to genotyping, the PCR products were mixed with markers which could be genotyped simultaneously in combinations of 4 μl of HEX, 1 μl of FAM and 1 μl of TET. Then 0.8 μl of the mixed PCR products was added to 4.5 μl of DNA size marker, centrifuged for 1 min at 1000 rpm and denatured using the PCR machine at a denaturing temperature of 94ºC for 9 mins. The denatured produ cts were subjected to electrophoresis and genotyping in an ABI 377 DNA Sequencer. A total of 82 informative microsatellite DNA markers was utilized for the genotyping (Table 1).

Traits analyzed: Offspring of the four sires born between 1997 and 2002 were evaluated for growth traits. Birth weight (BWT), weaning weight (WT6) and yearling weight (WT12) were measured in kg, while preweaning average daily gain (PREWADG) and postweaning average daily gain (POSTADG) were computed in kg/day.

QTL analysis: We adopted the methods of Knott et al. (1996), Haley and Knott (1992) and de Koning et al. (1998, 2001) for the detection and mapping of QTL in half -sib populations using least squares simple regression. We used the QTL Express computer program with a web -based user interface (http://qtl.cap.ed.ac.uk/) developed by Seaton et al. (2002) and based on the methods of the researchers mentioned above for the QTL analysis. The half -sib model of QTL Express run within and across sires, implemented the analysis in a two -step procedure: Firstly, microsatellite DNA marker data on progeny and their common parent (sire) were combined in a multi-point approach to calculate the probabilities of inheriting allele 1 or 2 from the sire at specific chromosomal intervals. These proba bilities were combined into coefficients with values between

0.0 and 1.0. Secondly, the phenotypic data on progeny were regressed on these coefficients in a within-common-parent regression analysis. A linear model containing the fixed effects of sire, sex, parity and season of birth as well as age as a covariate, was fitted to the probability coefficients and phenotypic data. Appropriate F-statistic thresholds for a P<0.05 chromosome-wise type 1 error rate were generated by permutation test as described by Churchill and Doerge (1994), Doerge and Churchill (1996) (and applied to other half-sib studies by Spelman et al. 1996 and Vilkki et al.1997). In determining significant thresholds, the QTL Express software (Seaton et al. 2002) computed both the F-statistics and the F-threshold at P<0.05 chromosome-wise level. QTL were classified as significant when the F-statistic exceeded the F-threshold indicating a marker-trait association. Finally, the boostrap procedure of Visscher et al. (1996) was followed to estimate confidence intervals of the QTL locations.

RESULTS

A scan for chromosomal regions of BTA1 segregating QTL influencing birth weight (BWT), weaning weight (WT6), yearling weight (WT12), preweaning (PREWADG) and postweaning (POSTADG) average daily gains was performed by genotyping paternal half-sib progeny of four Japanese Black sires. Table 1 shows the number of informative microsatellite markers, their intervals and positions across the four Japanese Black sires. It shows that in Sires 1, 2, 3 and 4, 18, 23, 11 and 19 markers spanning 135.1, 135.5, 125.8 and 135.5 cM respectively, were informative. The number of alleles ranged from a minimum of 3 to a maximum of 9.

Means and standard deviations of phenotypic data of the progeny across sires are shown in Table 2. Portrayed in Table 3 are estimated regression coefficients of sire QTL (additive/allele substitution) effects and estimated QTL locations corresponding to the peak of F-statistics as well as chromosome-wide 5% thresholds and 95% confidence intervals for the growth traits across sires. A QTL at 114 cM between the markers BMS4039 and BM3205 in Sire 2 achieved chromosome-wide significance (P<0.05). No other chromosomal regions were

shown to contain QTL effects that reached chromosome-wide significance. Figure 1 is a graphical depiction of the map of F-statistics in the four sires.

DISCUSSION

The early attainment of slaughter weight in beef cattle hinges on the growth potential of calves. Thus, weights at birth, weaning and yearling age as well as pre- and post-weaning average daily gain are important traits to the beef industry. However, the strong, positive genetic correlation of 0.5 - 0.58 between BWT and WT12 (Grosz and MacNeil, 2001) suggests that young breeding bulls selected for increased post-natal growth potential are expected to also sire calves having greater BWT resulting in increased risk of dystocia. On the other hand, selection of sires with genetic potential to reduce BWT would concurrently sacrifice the growth potential of calves. Therefore, identifying genes affecting pre- and post-natal growth coupled with marker assisted selection, has the potential to overcome this genetic antagonism by allowing selection for growth during specific developmental stages. This in effect, decreases both the incidence of dystocia and economic loss inherent to calving difficulty while minimizing any coincident effect on postnatal growth (Grosz and MacNeil, 2001).

The chromosome-wide significant ($P<0.05$) QTL influencing BWT detected at 114 cM between the microsatellite DNA markers BMS4039 and BM3205 within the confidence interval spanning 50 - 119.5 cM in Sire 2 (Table 3 and Figure 1) confirms the report of Stone et al. (1999) that BTA1 harbours putative QTL that significantly increased BWT in Brahman x Hereford cattle. They also found that other growth-related QTL for weaning and yearling weights reached suggestive thresholds only on BTA1. Similarly, Kim et al. (2003) in their study to detect QTL for growth and beef carcass fatness traits in Angus x Brahman cattle, reported a significant QTL affecting yearling weight in the interstitial region of BTA1. They also detected a total of 30 QTL with suggestive evidence for linkage and 4 other significant QTL on 19 chromosomes. Other researchers (Davis et al. 1998; Casas et al.1998; Keele et al. 1999;

Casas et al. 2000; Grosz and MacNeil 2001; MacNeil and Grosz 2002; Casas et al. 2003) have also investigated QTL affecting birth weight, growth and carcass composition of beef cattle in the USA. Grosz and MacNeil (2001) detected a QTL influencing birth weight at the telomeric end of BTA2 located at 114 cM in the interval between BM2113 and OarFCB11 microsatellite markers. Davis et al. (1998) reported significant QTL effects on birth weight in five chromosomes – BTA5, BTA6, BTA14, BTA18 and BTA21 located at 90, 48, 42, 116 and 4 cM respectively. MacNeil and Grosz (2002) detected QTL for liveweight on BTA17 located at 52 cM. Casas et al. 2003 detected putative QTL for birth weight on BTA1, BTA2 and BTA3, and for weaning weight on BTA29. Genotyping work in our laboratory is currently focussing on BTA2 and BTA5 as part of the on-going, long-term goal of whole genome-scanning of more Japanese Black cattle. The identification of a QTL herein, indicates the presence of detectable segregation of alleles, hence a significant variation affecting an important trait (BWT) in Japanese Black cattle. This would be useful for the implementation of marker-assisted selection as an effective tool for the early attainment of slaughter weight in Wagyu cattle. Furthermore, this finding could pave the way for comparative candidate positional cloning in Japanese Black cattle using ghrelin growth hormone secretagogue -receptor, glycogenin or Pit-1 as candidate genes. Subsequent prospects of isolating and characterising the genes using single nucleotide polymorphisms (SNPs) appear quite promising.

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Table 1. Informative microsatellite DNA markers used for genotyping in the 5 Japanese Black cattle families and their relative map positions (cM)*

Family	Marker	Position	Family	Marker	Position	Family	Marker	Position	Family	Marker	Position
1	BMS1928	6.9	2	BM8139	8.2	3	BMS2321	14.0	4	BMS1928	6.9
1	BMS711	21.3	2	TGLA57	46.2	3	ILSTS104	28.2	4	BMS711	21.3
1	ILSTS104	28.2	2	BMS4012	51.0	3	BMS4002	47.9	4	TGLA57	46.2
1	MB055	32.0	2	BMS4013	61.3	3	BMS4012	51.0	4	BMS4035	55.0
1	TGLA57	46.2	2	BMS4001	64.7	3	BMS4035	55.0	4	BMS4029	61.3
1	BMS4012	51.0	2	BM9019	67.5	3	RME36	63.0	4	BM9019	67.5
1	BMS4035	55.0	2	BL26_1	77.7	3	BM8246	76.2	4	BMS4008	71.7
1	RM326	55.6	2	BMS4006	79.4	3	BMS119	88.6	4	BMS4048	76.2
1	RME36	63.0	2	URB038	80.6	3	BMS4019	98.8	4	URB038	80.6
1	INRA049	67.5	2	MCM130	83.3	3	UWCA46	113.8	4	BMS4010	87.1
1	BM65O6	69.2	2	BMS4010	87.1	3	BMS599	125.8	4	BM864	88.2
1	URB038	80.6	2	BM864	88.2				4	BMS1170	92.8
1	BMS4052	94.6	2	BMS1170	92.8				4	BMS4019	98.8
1	BMS4028	95.6	2	BMS4028	95.6				4	BMS4011	102.1
1	BMS4040	98.8	2	BMS4019	98.8				4	BMS4049	114.3
1	BMS1789	100.9	2	BMS1789	100.9				4	BMS918	118.1
1	BMS4044	128.7	2	BMS1939	104.1				4	BMS599	125.8
1	BMS2263	135.1	2	BMS4039	108.3				4	BMS4044	128.7
			2	BM3205	113.8				4	BMS922	135.5
			2	BMS599	125.8						
			2	BMS4043	128.7						
			2	BMS2263	135.1						
			2	BMS4014	135.5						
Total	18			23			11			19	

*Based on the bovine genetic map at the U.S. Meat Animal Research Centre (Kappes et al., 1997; http://sol.marc.usda.gov)

Table 2. Distribution of progeny across sires, sexes and means ± standard deviations of growth traits of Japanese Black cattle

Sire	Males	Females	Total	Birth weight (kg)	WT6 (kg)	WT12 (kg)	PREWADG (kg/day)	POSTWADG (kg/day)
1	22	18	40	34.73 ± 4.70	163.92 ± 21.73	300.85 ± 31.80	0.72 ± 0.11	0.76 ± 0.18
2	24	12	36	33.97 ± 4.75	172.62 ± 19.38	284.67 ± 34.14	0.77 ± 0.09	0.70 ± 0.19
3	10	9	19	28.68 ± 5.13	177.63 ± 20.52	299.95 ± 33.55	0.83 ± 0.10	0.62 ± 0.14
4	8	9	17	28.71 ± 3.32	176.71 ± 22.94	283.59 ± 29.12	0.82 ± 0.12	0.56 ± 0.20

Table 3. Regression coefficients of sire QTL (allele substitution) effects (ß±S.E.), estimated QTL locations and chromosome-wide F-statistics for growth traits of Japanese Black calves

Sire Family	1	2	3	4
ß±S.E.	Birth weight (BWT)			
	-3.57±1.96	3.70±1.58	3.10±2.43	-4.43±1.24
Estimated QTL location (cM)	61	114*	47	111
F-statistics	3.32ns	5.49 significant	1.63ns	12.71ns
Likelihood Ratio	3.07	4.84	1.41	5.00
F-threshold (P<0.05)	9.1	5.41	11.69	17.30
95% Conf. Interv (cM)	40.0-119.0	50.0-119.5	40.0-125.0	40.0-126.0
	Weaning weight (WT6)			
ß±S.E.	-15.41±9.40	15.59±7.92	-15.97±8.86	39.04±11.33
Estimated QTL location (cM)	14	18	125	59
F-statistics	2.69ns	3.87ns	3.24ns	11.87ns
Likelihood Ratio	2.51	3.51	2.62	4.82
F-threshold (P<0.05)	9.11	10.19	13.59	30.22
95% Conf. Interv (cM)	4.5-124.0	13.5-132.0	40.5-125.0	40.0-129.5
	Yearling weight (WT12)			
ß±S.E.	-21.40±14.45	-34.82±15.12	-18.49±15.82	-55.35±21.87
Estimated QTL location (cM)	14	48	52	115
F-statistics	2.19ns	5.31ns	1.37ns	6.40ns
Likelihood Ratio	2.06	4.69	1.20	3.35
F-threshold (P<0.05)	9.31	6.22	12.63	20.37
95% Conf. Interv (cM)	6.5-122.0	40.0-132.0	40.0-123.0	42.0-131.0
	Preweaning average daily gain (PREWADG)			
ß±S.E.	-0.09±0.05	0.07±0.04	-0.09±0.04	0.20±0.06
Estimated QTL location (cM)	14	18	125	59
F-statistics	4.09ns	3.61ns	4.43ns	11.70ns
Likelihood Ratio	3.72	3.29	3.40	4.79
F-threshold (P<0.05)	4.25	4.43	12.94	32.16
95% Conf. Interv (cM)	13.0-122.0	17.0-132.0	40.0-125.0	50.0-130.0
	Postweaning average daily gain (POSTADG)			
ß±S.E.	0.10±0.09	-0.21±0.10	-0.14±0.06	0.18±0.06
Estimated QTL location (cM)	82	27	29	77
F-statistics	1.25ns	4.81ns	5.20ns	9.44ns
Likelihood Ratio	1.20	4.29	3.87	4.24
F-threshold (P<0.05)	9.09	5.88	6.05	17.22
95% Conf. Interv (cM)	20.0-128.0	20.0-132.0	20.0-125.0	20.0-132.0

* P chromosome-wide significance (P<0.05) level QTL. ns=not significant. Confidence intervals based on the reduced model only.

Figure 1. Map of F-statistics in the 4 Japanese Black sire families. In Sire 2 family, significant (bold line) P<0.05 chromosome-wide threshold for BWT is depicted.